Neurotoxicity and Pharmacokinetics of Intrathecal Perfusion of ACNU in Dogs

Masato Kochi,2 Jun-ichi Kuratsu, Yosuke Mihara, Shu-ichi Takaki, Nobuhiro Inoue, Nobuyuki Sueyoshi, Shozaburo Uemura, and Yukitaka Ushio

Department of Neurosurgery, Kumamoto University Medical School, l-I-I Honjo, Kumamoto 860, Japan

ABSTRACT

To test the feasibility of intrathecal perfusion of ACNU (3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride) in the treatment of subarachnoid dissemination of malignant glioma, the neurotoxicity and pharmacokinetics of ACNU were studied in dogs. ACNU (1-2 mg dissolved in 10-20 ml of lactated Ringer's solution or artificial cerebrospinal fluid (CSF)) was administered via the right lateral ventricle by constant drip infusion and CSF was drained by lumbar puncture. The infusion time was from 15 to 71 min. For the control, a bolus injection was given. No neurological and systemic symptoms were noted after perfusion. Histological examination of the brain and spinal cord revealed only mild denudation of ependyma in the wall of the ventricles in a dog treated three times with 2 mg ACNU (perfusion twice, bolus injection once) and in 2 dogs perfused with 1 mg ACNU once a week for 10 weeks. ACNU was not detected in lumbar CSF after bolus injection into the lateral ventricle. When 1 mg of ACNU, dissolved in 10 ml of artificial CSF, was perfused for a duration of 22 to 31 min, it started to appear in the lumbar CSF 10 to 15 min after the start of perfusion, reaching a maximum concentration of 13.88 to 22.31 μg/ml. The area under the drug concentration-time curve was 344 to 706 μg x min/ml; the half-time was 15.5 to 19.5 min. The distribution volume was 30.6 to 54.1 ml. These findings suggest the feasibility of intrathecal perfusion of ACNU in the treatment of patients with subarachnoid dissemination of glioma.

INTRODUCTION

Recent advances in the treatment of malignant glioma have increased the life expectancy of these patients; however, subarachnoid dissemination (meningeal gliomatosis), a serious complication of malignant glioma, has also increased (1-4). Radiotherapy and systemic chemotherapy appear to be ineffective. Intrathecal chemotherapy with methotrexate (5-11), 1-beta-D-arabinofuranosylcytosine (6, 7, 12), thio-TEPA (6, 8, 13, 14), bleomycin (15), or neocarzinostatin (16-19) has been tried; however, the effect of these drugs was only temporary (20).

ACNU,1 one of the chlorothymlnitrosoureas developed in Japan, has proved to be effective against malignant glioma and at present it is the drug of choice in Japan in the treatment of malignant glioma (21, 22). We studied the intrathecal use of this drug (23-25) and demonstrated that in experimental animals, diffusional transport of ACNU into the brain parenchyma was minimal when it was administered into the subarachnoid space, that the intrathecal administration of a small dose of ACNU produced no side effects, and that intrathecal administration of ACNU significantly prolonged the survival time of rats with subarachnoid dissemination of Walker 256 tumors. A phase I study revealed that doses of ACNU lower than 10 mg could be administered safely via the lateral ventricle (26); however, when the drug was administered via the lateral ventricle, it failed to reach the distant subarachnoid space such as the lumbar region (26). We posit that degradation of ACNU is rapid in the CSF and that clearance of ACNU from the CSF space into the blood vessels is rapid because of its high vascular permeability.

To utilize the advantages and overcome the disadvantages of ACNU, we considered administering ACNU by perfusion instead of infusion in the treatment of subarachnoid dissemination of glioma (26). In the study presented here, we examined the neurotoxicity and intrathecal pharmacokinetics of ventriculolumbar perfusion of ACNU in dogs.

MATERIALS AND METHODS

Five adult mongrel dogs, 3 to 4 years old and weighing 8.5 to 10.5 kg, were used. They were anesthetized with ketamine and pentobarbital and placed in a sphinx position. Anesthesia was maintained with a mixture of nitrous oxide, oxygen, and halothane. Using two ear pins and two infraorbital pins, the head was placed in a head holder in such a way that the orbitomeatal plane was horizontal. A burr hole was made 1 cm anterior to the ear line and 1 cm to the right of the midline; ventricular puncture was performed vertically. A Pudenz ventricular catheter was placed after CSF reflux was confirmed and lumbar puncture was performed. For the repeated administration of ACNU, the Ommaya system was placed; it consists of a reservoir under the scalp and a connected catheter in the right lateral ventricle. ACNU (0.1 or 0.05 mg/ml) was dissolved in lactated Ringer's solution or artificial CSF and administered to the lateral ventricle at a concentration of 1 to 2 mg/10 to 20 ml of vehicle during a period of 15 to 71 min. (When 1 mg of ACNU was dissolved in 10 ml of artificial CSF, the pH was 7.40.) During ACNU administration, the CSF was drained by lumbar puncture. In one dog, ACNU (2 mg dissolved in 2 ml of water) was administered into the lateral ventricle by bolus injection and the CSF was sampled by lumbar puncture. ACNU was provided by Sankyo Co., Tokyo.

To assess systemic and local toxicity, body weight changes, behavioral changes, and neurological symptoms were recorded daily. Routine CSF examination was performed before and periodically after the perfusion. Hemograms and blood chemistry examinations were also performed. Three weeks after the last administration of ACNU the animals were sacrificed, the brain and spinal cord were removed and fixed in 10% formalin, and representative sections were stained with hematoxylin and eosin and Klüver-Barrera. One dog was not perfused and served as a control. Areas inspected under the microscope included the subarachnoid pia, cerebral gray matter, cerebral white matter, caudate nucleus, putamen, globus pallidus, thalamus, hypothalamus, fornices, ependymal surface, choroid plexi, midbrain, pons, medulla oblongata, cerebellum, spinal cord, and spinal nerves.

To assess the intrathecal pharmacokinetics, lumbar CSF was collected serially at intervals of 1 to 23 min from the start until 60 to 168 min after the cessation of perfusion; the ACNU concentration was assayed by high performance liquid chromatography (27). The specimens (CSF and blood) were frozen instantaneously after sampling to prevent the decomposition. The drug was extracted in 1,2-dichloroethane under cold condition using amber colored glass vessels. The drug was separated using a reversed phase column with the ion-pair partition technique. The eluted drug was detected by absorption at 254 nm, and the quantity was estimated from its peak height. The detection limit of ACNU was 0.04 μg/ml. The ACNU concentration in blood was also measured serially. In the dog who received 2 mg of ACNU dissolved in 2 ml of bolus injection into the right lateral ventricle, CSF was sampled by lumbar puncture at 30, 60, 90, and 120 min postinjection.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: ACNU, 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(chloroethyl)-1-nitrosourea; CSF, cerebrospinal fluid; AUC, area under the drug concentration-time curve.

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RESULTS

Neurotoxicity. No loss of appetite, no decrease in body weight, and no neurological signs were observed during or after intrathecal perfusions.

CSF examinations including cell count, glucose content, and electrolytes were all within the normal range except for a slight elevation of protein (70 mg/ml on average). Hemograms and blood chemistry analysis were within the normal range after 10 intrathecal perfusions of 1 mg ACNU.

Pathological examination of the brain and spinal cord of a dog treated with intraventricular administration of 2 mg ACNU (perfusion twice, bolus injection once) and sacrificed 3 weeks after the last treatment revealed only denudation of ependyma in a small area of the third and lateral ventricles; the ependymal lining was relatively well preserved. No ependymitis, periventriculitis, meningitis, vasculitis, or demyelination was noted.

Two dogs treated with intrathecal perfusion of 1 mg ACNU dissolved in artificial CSF once a week for 10 consecutive weeks were sacrificed 3 weeks after the last treatment. Macroscopic examination of the brain showed mild dilation of the lateral ventricles in one dog. Pathological examination showed denudation of ependyma in small areas (Fig. 1); however, most of the ependymal lining was relatively well preserved (Fig. 2).

Intrathecal Pharmacokinetics. No ACNU was detected in lumbar CSF after the intraventricular bolus injection of 2 mg ACNU; however, various levels of ACNU were detected in lumbar CSF during and after ventriculolumbar perfusion.

When ACNU (1 or 2 mg dissolved in 10 or 20 ml of lactated Ringer's solution) was administered over a period of 15 to 71 min, it was detectable in lumbar CSF 5 to 13 min after the start of perfusion, reaching the highest concentration 17 to 65 min after the start of perfusion. When 1 mg ACNU dissolved in 10 or 20 ml was administered for 31 and 37 min, the maximum concentration was 6.26 and 12.63 µg/ml, and the AUC was 346 and 444 µg x min/ml, respectively (Table 1). The maximum concentration and AUC increased as the infusion volume increased (Fig. 3).

When 2 mg of ACNU dissolved in 20 ml of lactated Ringer's solution were administered over period of 22, 45, or 71 min, the maximum concentration was 25.76, 16.12, and 7.62 µg/ml, and the AUC was 896, 840, and 462 µg x min/ml, respectively (Table 1, Fig. 4A). When 1 mg of ACNU dissolved in 10 ml of lactated Ringer's solution was administered for 15 and 31 min, the maximum concentrations were 22.26 and 6.26 µg/ml, and the AUCs were 529 and 346 µg x min/ml, respectively. These data indicate that the maximum concentration and AUC decreased as infusion time increased.

The elimination phase of ACNU in lumbar CSF followed linear kinetics (Fig. 4B) and the half-time was 18 min on average (Table 1).

Using the one-compartment model, the ACNU concentration in lumbar CSF during perfusion is

\[ C'_t = \frac{R_{inf}}{V_d \cdot K_e} \left( 1 - e^{-K_e \cdot t} \right) \]

where \( C'_t \) is concentration in CSF (µg/ml), \( R_{inf} \) is infusion rate (µg/min), \( K_e \) is elimination constant (min\(^{-1}\)), and \( V_d \) is volume of distribution (ml) (28). Using this formula, \( V_d \) was calculated as 29.6 to 125.8 ml (Table 1).

The serum concentration of ACNU did not exceed 0.10 µg/ml, even when the concentration in the lumbar CSF was at its highest (25.37 µg/ml (Fig. 5)).

When 1 mg of ACNU dissolved in 10 ml of artificial CSF was administered over a period of 22 to 31 min, the ACNU concentration in the lumbar CSF reached the maximum, 13.88 to 22.31 µg/ml in 26 to 35 min. The AUC was 344 to 706 µg x min/ml, the half-time was 15.5 to 19.5 min (17.4 min on
The ideal drug for intrathecal use should have the following characteristics: a rapid rate of mixing and distribution within the various CSF compartments; a slow rate of drug clearance from the CSF; and a short time course of drug action (29, 30). ACNU has a high capillary transfer constant and its clearance from CSF is rapid. This characteristic is not desirable in terms of the therapeutic effect but is desirable in terms of neurotoxicity (23). ACNU is cell cycle nonspecific and its time course of action is short.

We studied the toxicity and therapeutic effect of intrathecal ACNU in rats with leptomeningeal tumors and found that the drug could be safely administered intrathecally when less than 1.5 mg/kg was used (24, 25). Levin et al. (31) who studied the central nervous system toxicity and CSF pharmacokinetics of intraventricular chloroethyl nitrosoureas in dogs reported that ACNU was tolerated at doses of 0.2 to 0.8 mg/week for 8 consecutive weeks and that ependymitis and periventriculitis increased with increases doses of ACNU.

We encountered no neurotoxicity or systemic toxicity in the dogs perfused with 1 mg of ACNU dissolved in 10 ml of artificial CSF once a week for 10 consecutive weeks. Denudation of the ependyma in small areas was seen; however, this was present even in dogs that received intrathecal saline injection and was not considered specific to ACNU per se (31).

Geiser et al. (32) reported that the toxic effect of intrathecal chemotherapy in children with acute leukemia was significantly reduced by the use of Elliot's B solution. Therefore, we used artificial CSF as the ACNU vehicle.

Because CSF elimination of ACNU is rapid and the time for drug diffusion from the ventricle to the lumbar and cerebral convexity-subarachnoid space is slow, it is possible that a significant amount of ACNU would hydrolytically decompose before reaching the distant CSF space (31). Rieselbach et al. (33), who studied the subarachnoid distribution of radioisotope ACNU in rats with leptomeningeal tumors and found that the drug could be safely administered intrathecally when less than 1.5 mg/kg was used (24, 25).

Table 1 Pharmacokinetic variables following the intrathecal perfusion of ACNU dissolved in lactated Ringer's solution

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Volume (ml)</th>
<th>Infusion time (min)</th>
<th>Drainage (ml)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$t_\text{d}$ (min)</th>
<th>AUC (µg x min/ml)</th>
<th>$K_d$ (min$^{-1}$)</th>
<th>$V_d$ (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>15</td>
<td>24.5</td>
<td>22.26</td>
<td>10</td>
<td>529</td>
<td>0.069</td>
<td>36.1</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>31</td>
<td>27.4</td>
<td>6.26</td>
<td>39</td>
<td>346</td>
<td>0.018</td>
<td>125.8</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>37</td>
<td>37.1</td>
<td>12.63</td>
<td>10.5</td>
<td>444</td>
<td>0.066</td>
<td>29.6</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>22</td>
<td>14.4</td>
<td>25.76</td>
<td>21.5</td>
<td>896</td>
<td>0.032</td>
<td>55.6</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>45</td>
<td>16.12</td>
<td>25.76</td>
<td>17</td>
<td>840</td>
<td>0.041</td>
<td>56.8</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>71</td>
<td>33.9</td>
<td>7.62</td>
<td>10.5</td>
<td>462</td>
<td>0.066</td>
<td>55.5</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ maximum concentration.

Table 2 Pharmacokinetic variables following the intrathecal perfusion of 1 mg of ACNU dissolved in 10 ml of artificial CSF

<table>
<thead>
<tr>
<th>Infusion time (min)</th>
<th>Drainage (ml)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$t_\text{d}$ (min)</th>
<th>AUC (µg x min/ml)</th>
<th>$K_d$ (min$^{-1}$)</th>
<th>$V_d$ (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>21.4</td>
<td>18.07</td>
<td>19.5</td>
<td>538</td>
<td>0.036</td>
<td>40.8</td>
</tr>
<tr>
<td>25</td>
<td>22.4</td>
<td>22.31</td>
<td>15.5</td>
<td>706</td>
<td>0.044</td>
<td>30.6</td>
</tr>
<tr>
<td>26</td>
<td>18.2</td>
<td>13.88</td>
<td>16</td>
<td>353</td>
<td>0.043</td>
<td>43.2</td>
</tr>
<tr>
<td>27</td>
<td>17.2</td>
<td>14.71</td>
<td>17</td>
<td>344</td>
<td>0.041</td>
<td>49.4</td>
</tr>
<tr>
<td>31</td>
<td>17.7</td>
<td>13.90</td>
<td>19</td>
<td>357</td>
<td>0.036</td>
<td>54.1</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$ maximum concentration.
Fig. 4. ACNU concentration in lumbar CSF during and after infusion of 2 mg of ACNU dissolved in 20 ml of lactated Ringer's solution at three different infusion times (top, 22 min; middle, 45 min; bottom, 71 min). In A, the maximum concentrations were 25.76, 16.12, and 7.62 µg/ml, and AUCs were 896, 840 and 462 µg x min/ml, respectively. These findings showed that the maximum concentration and AUC decreased as the infusion time increased. B, semilog plot. The elimination phases followed linear kinetics and the half-times were 21.5, 17, and 10.5 min, respectively.

Fig. 5. ACNU concentration in lumbar CSF and serum during and after 22 min infusion of 2 mg of ACNU dissolved in 20 ml of lactated Ringer's solution. The maximum concentration of ACNU in serum was 0.10 µg/ml, even when the concentration in the lumbar CSF was at its highest (25.37 µg/ml).

perfusion as an approach to improve the therapeutic index of intrathecal chemotherapy (34).

Our experiments showed that adequate CSF distribution of ACNU was achieved by ventriculolumbar perfusion but not by intraventricular bolus injection. When 1 mg of ACNU dissolved in 10 ml of lactated Ringer's solution was perfused, the AUC values ranged from 346 to 529 µg x min/ml. We did not use artificial CSF at the beginning of these experiments because decomposition of ACNU in this vehicle is rapid (23% of ACNU is decomposed in artificial CSF in 60 min). However, we use it in the experiments where the perfusion time was short enough to maintain ACNU mostly in its active form. When 1 mg of ACNU dissolved in 10 ml of artificial CSF was perfused, the AUC values ranged from 344 to 706 µg x min/ml, similar to those obtained with lactated Ringer's solution as the ACNU vehicle. In vitro, against 9L gliosarcoma cells, 300 µg x min/ml ACNU achieve a 1.5-log cell kill and 380 µg x min/ml a 3-log cell kill, and against a human glioma cell line, HU-126, 240 µg x min/ml achieves a 1.5-log cell kill (31). Thus, we expect that, if humans can tolerate ACNU in the CSF at concentrations comparable to those tolerated by dogs, ACNU delivered by ventriculolumbar perfusion would have a strong cytotoxic effect on neoplastic cells dispersed within, or lining, the CSF compartments. In addition, repeated doses would further enhance this cytotoxicity. The distribution volumes ranged from 30.6 to 54.1 ml when 1 mg of ACNU dissolved in 10 ml of artificial CSF was administered for 22 to 31 min and these values are compatible with local (intrathecal) chemotherapy. Furthermore, the maximum concentration and AUC could be controlled by adjusting the infusion volume and infusion time; the maximum concentration and AUC increased as infusion volume increased, and the maximum concentration and AUC decreased as infusion time increased. Further studies are under way to establish the most appropriate regimen of intrathecal ACNU perfusion, to treat patients with subarachnoid dissemination of malignant glioma.

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